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THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

November 26, 1952

Dr. Joshua Lederberg
University of Wisconsin
College of Agriculture
Department of Genetics
Madison 6, Wisconsin

Dear Dr. Lederberg:

The problem you raised in your letter of November 20, has been a puzzle to us for some time and there is no good explanation available at present. The facts as I see them at present are these:

(1) On the average only about 70% of the inoculated virus is adsorbed, i.e. 30% of the virus remains detectable in the allantoic fluid of the injected eggs. With "excessive doses" of virus less may be adsorbed.

(2) In experiments with ultraviolet, inactivated interfering virus adsorption is of the same order. If the subsequently injected dose of active challenge virus is small additional adsorption occurs but the degree of adsorption appears to be very small. If the challenge dose is large so that the hemagglutination test can be used for evaluation, the degree of adsorption again is of the order of 70%. This difference may be due to the variable chances of "effective contact" with cell receptors, and the larger the interfering dose the more cell receptors may be occupied or destroyed and the chances that the challenge virus makes contact with the remaining receptors increases with the quantity used. One also may consider that an equilibrium is established between adsorbed and free virus. A large challenge dose upsets this equilibrium, a small dose hardly affects it. These experiments actually exclude your suggestion that the cells are "conditioned".

(3) Allantoic fluid contains an inhibitor of hemagglutination considered to be "receptor substance" which combines with the virus but the virus is capable of eluting again. However, according to Hardy and Horsfall a certain number of virus particles remain permanently attached to the inhibitor. However, if adsorption were thus prevented, it should also be inhibited on passage.

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(4) The conditions for contact of virus with host cells in the allantois exposed to influenza virus differ, of course, to some extent from those obtained in the phage-bacterium system. In the latter case cells and virus are intermixed, whereas in the former system the virus has to reach the fluid-tissue interface in order to be adsorbed. That, too, could conceivably affect the results of adsorption.

I know it is always a sort of a shock to the phage worker when he realizes the technical difficulties and the inherent inaccuracies encountered in animal virus work. The titration technics are reproducible only within relatively broad limits and, consequently, one never can be too assured about any interpretations given. Some adsorption experiments have been done in recent weeks in which another approach was used. In that case the embryos were infected at t_o and some were then de-embryonated at 5, 15, 30, 60, 120 and 180 minutes. In other words adsorption was interrupted at these time intervals. The yield of virus liberated from the membranes into the medium at 10 hours increased up to the 1 hour-series quite markedly and only slight increases were noted thereafter up to the 3-hour series. However, in this instance one has to consider that the longer the eggs are kept intact the more undisturbed the early intracellular events. Nevertheless, these data imply that by the 1st hour adsorption is practically complete and additional adsorption, if it really occurs, is rather slight.

I am afraid these are all the thoughts I have at present, and that they do not answer quite your questions.

With kindest regards, I am

Sincerely yours,

Werner Henle

Werner Henle, M. D.

WH/dm